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RESEARCH ARTICLE

Diversity and distribution of ectoparasites infesting *Podiceps cristatus* and *Tachybaptus ruficollis* (Aves, Podicipediformes) at a Ramsar site in northeast Algeria: new records

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Abstract

Algeria hosts 55 Ramsar sites of international significance, serving as crucial habitats for a wide range of migratory, wintering, and breeding waterbird species. Despite this, research on the ectoparasites of aquatic avifauna in Algeria remains limited. This study aims to investigate the ectoparasite diversity infesting the Little Grebe (*Tachybaptus ruficollis* Pallas, 1764) and the Great Crested Grebe (*Podiceps cristatus* Linnaeus, 1758) in northeastern Algeria. To do so, we examined grebes of both species at Lake Tonga between 2022 and 2023.

A total of 8,057 ectoparasites were collected, 24 of which belonged to Phthiraptera and 8,033 to Acari. Two lice species were identified: *Aquanirmus podicipis* Denny, 1842, belonging to the suborder Ischnocera, infesting the Great Crested Grebe and *Pseudomenopon dolium* Rudow, 1869, from the suborder Amblycera, infesting the Little Grebe. As for Acari, we identified two mite species belonging to the suborder Astigmata (Pterolichoidea; Ptiloxenidae): *Ptiloxenus major* Mégnin & Trouessart, 1884, and *Ptiloxenus colymbi* Canestrini, 1878 the latter of which had not been reported since its original description. This study reports a new host-parasite association in the global mite fauna (Great Crested

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Grebe - *Ptiloxenus colymbi* Canestrini, 1878). All the identified ectoparasite species are reported for the first time in Algeria.
Feather mite infestation did not differ significantly between breeding and wintering seasons and showed only a weak correlation with host morphometric parameters. These findings suggest that neither season nor body size strongly influence feather mite dynamics in the studied grebes, while still providing valuable insights into their host–parasite relationships.

Keywords
Feather mites, Great Crested Grebe, Lake Tonga, lice, Little Grebe.

Introduction

The symbiotic relationship between wild birds and parasites, including mites, ticks, lice, and fleas has been a subject of considerable interest in ecological research (Clayton et al. 2010). Ectoparasites can serve as vectors for a wide range of avian diseases, transmitting parasitic, rickettsial, bacterial, and viral infections between domestic and migratory birds. Additionally, they can cause allergic reactions, irritation, anorexia, reduced productivity in affected birds, and myiasis, all of which can impact the health and fitness of avian populations (Zumpt 1965; Dik 2006). Understanding the interactions between birds and their ectoparasites is essential for comprehending broader ecological dynamics, as these relationships can significantly influence both host populations and parasite communities. Furthermore, this knowledge has implications for wildlife conservation and management, given that ectoparasites are integral to a larger ecological network involving multiple host species.

Situated within the Mediterranean basin, Algeria plays a crucial role in avian migration, wintering, and breeding. The country hosts 55 Ramsar sites, more than any other African nation, providing vital habitats that attract large populations of waterbirds on a regular basis (Isenmann and Moali 2000; Samraoui and Samraoui 2008; Samraoui et al. 2011).

In northeastern Algeria, avian populations have been documented to include 61 species across 17 families and 44 genera. Among these, the family *Podicipedidae* (grebes) is represented by three species, all classified as Least Concern (LC) in terms of conservation status (BirdLife International. 2018, 2019a,b; Gherib et al. 2021).

The Little Grebe (*Tachybaptus ruficollis*) and the Great Crested Grebe (*Podiceps cristatus*) are resident breeders at Lake Tonga (Loucif et al. 2020; Mecif and Boucheker 2020). These species are recognized as excellent bioindicators of the structure and functioning of aquatic ecosystems (O'Donnell and Fjeldså 1997; Fjeldså 2004).

However, despite their ecological significance, relatively few studies have examined avian parasites in Algeria. Existing research has primarily focused on the parasite diversity of domestic birds (Meguini et al. 2018; Boucheikhchoukh et al. 2023) and a limited number of wild species, including the Common Coot (*Fulica atra*) (Rouag-Ziane et al. 2007), the Greater Flamingo (*Phoenicopterus roseus*) (Touati and

Samraoui 2013), the Glossy Ibis (*Plegadis falcinellus*) (Touati et al. 2015), the Little Egret (*Egretta garzetta*) (Temimi et al. 2017), and the White Stork (*Ciconia ciconia*) in both urban and natural habitats (Bouguessa-Cheriak et al. 2017; Touati et al. 2022). Other studies have addressed parasitic infestations in the Cattle Egret (*Bubulcus ibis*) (Abdessamed et al. 2018), the Common Moorhen (*Gallinula chloropus*) (Ziani et al. 2021), the migratory European Turtle Dove (*Streptopelia turtur*), the exotic Eurasian Collared Dove (*Streptopelia decaocto)* (Absi et al. 2021), and the Black-headed Gull (*Chroicocephalus ridibundus*) (Hamdoune et al. 2023).

Given the scarcity of research on the parasitic fauna of birds in Algeria, the present study is of significant importance, as it provides an opportunity to identify previously unrecorded species of lice and feather mites affecting the resident avifauna in the eastern regions of the country. This study focuses specifically on two grebe species inhabiting Lake Tonga. The presence of diverse avian communities comprising wintering, nesting, and migratory stopover species creates a dynamic environment conducive to parasite exchange. Identifying these ectoparasites is essential for advancing our understanding of parasite-host interactions within established ecological communities.

Materials and Methods

Study area

The study was conducted from March 2022 to September 2023 at Tonga Lake (36° 53' N, 08° 31' E), a Biosphere Reserve and Ramsar site within El Kala National Park in northeastern Algeria (Fig. 1 and 2). With an area of 2,600 ha, this freshwater marsh is considered the most important wintering and nesting site in North Africa, hosting more than 25,000 aquatic birds. It serves as a habitat for rare, vulnerable, or declining avian species, such as the White-headed Duck (*Oxyura leucocephala*), Marbled Teal (*Marmaronetta angustirostris*), Ferruginous Duck (*Aythya nyroca*), and Purple Swamphen (*Porphyrio porphyrio*) (Lazli et al. 2011, 2012; Gherib and Lazli, 2016,2017; Mecif and Boucheker 2020). The study region has a typical Mediterranean climate, characterized by warm to hot, dry summers and mild to cool, wet winters (Boumezbeur 1993).

Parasites preparation and examination

A total of 32 grebes (13 Great Crested Grebes and 19 Little Grebes) from Lake Tonga were examined. Of these, 29 were captured using mist nets and visually inspected in complete conformity with ethical guidelines for handling wild birds. Each bird was handled in full compliance with previously established protocols (Gaud and Atyeo 1996; Clayton and Drown 2001). They were measured and visually inspected for ectoparasites in less than five minutes to minimize stress. After that, all birds were released back into the wild.

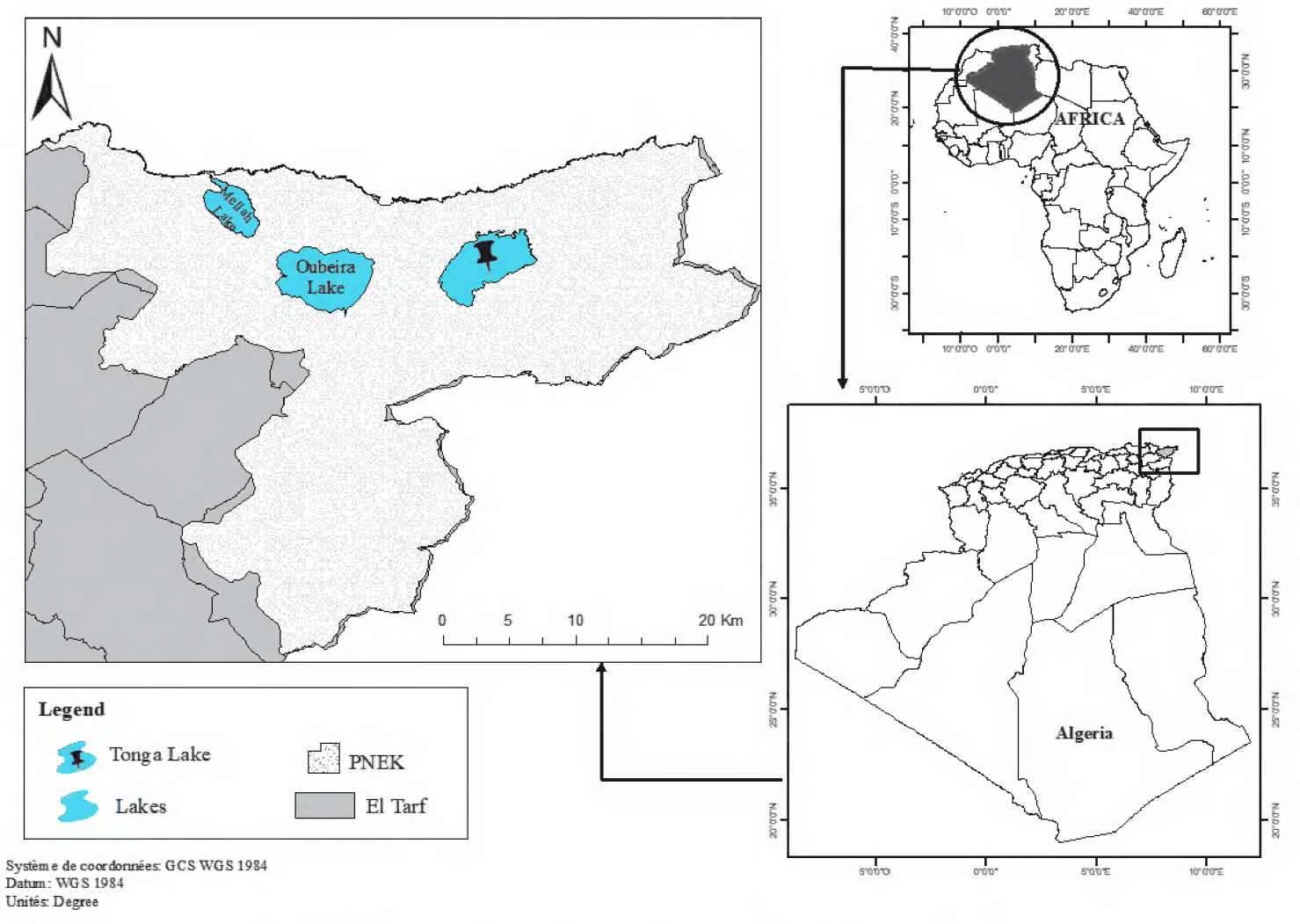

Figure 1. Location of the study site in El Kala National Park.

Figure 2. View of Lake Tonga.

National Park officers recovered three dead birds, which were sent to Chadli Bendjedid El Tarf University for examination. Each specimen was stored in plastic bags and frozen to prevent the loss of feathers and ectoparasites. Later, they were examined for ectoparasites as recommended by previous protocols (Clayton and Drown 2001; Dik 2014). Subsequently, lice and feather mite specimens were collected manually using fine-point tweezers and preserved in tubes containing 70% ethanol.

Prior to identification, lice specimens were cleared in 10% potassium hydroxide (KOH) for 24 hours, rinsed in distilled water, and passed through an - ethanol gradient series (70% and 99%) for two consecutive days. Then, they were mounted in Canada balsam on glass slides and identified based on their morphological characteristics and dichotomous keys (Edwards 1965; Price 1974; Palma 1978; Price et al. 2003; Dik 2014) using a Leica DM750 binocular light microscope (Leica Microsystems GmbH, Wetzlar, Germany). The specimens were photographed using a Leica DFC295 digital microscope camera (Leica Microsystems, Wetzlar, Germany) at Selçuk University, Konya, Türkiye.

For Astigmata, a representative number of mites from collected samples were cleared for 48 hours with lactophenol and then mounted on microscope slides in Hoyer's medium (Evans 1992). They were then identified by Dr. Ioana Cristina Constantinescu in the "Grigore Antipa" National Museum of Natural History, Bucharest, Romania.

Scanning electron microscopy

The feather mites were fixed with 3% glutaraldehyde in sodium cacodylate buffer (0.1M, pH 7.4) and stored in a refrigerator. The specimens were then dehydrated with ethanol solutions (30%, 50%, 70%, 80%, 90%, and 100%) and were then transferred to a mixed solution of ethanol and hexamethyldisilazane (HMDS) (3:1, 1:1, and 1:3), and finally in 100% HMDS. (Han et al. 2019; Han and Min 2019).The samples were left overnight for chemical evaporation and complete drying. The probes were mounted on aluminum stubs covered with conductive double-sided adhesive carbon tabs and then sputter-coated (SEM Coating Unit E5100) with gold for 60 seconds. The specimens were analyzed and photographed using a Phenom Pro desktop SEM (Thermo Fisher Scientific, The Netherlands) at 10kV acceleration voltage.

Data analysis

The following parasitological parameters were evaluated: (1) prevalence, defined as the proportion of individuals within a host taxon that is infested, calculated using the formula $P(\%) = n / N \times 100$, where n represents the number of infected birds and N denotes the total number of examined birds; (2) mean intensity, referring to the number of mites or chewing lice per host among infested individuals; and (3) mean abundance, representing the number of mites or chewing lice per host among all examined individuals (Margolis et al. 1982).

Statistical analyses were performed using R software (version 4.4.0; R Core Team, 2024), with the Rcmdr and FactoMineR packages. Data exploration was applied

using boxplots to assess variability and distribution patterns, the Shapiro–Wilk test to evaluate normality, and Student's t-tests to compare the prevalence and seasonal variation of feather mites on the host birds. In addition, a Principal Component Analysis (PCA) was performed to examine the relationships between morphometric parameters and feather mite abundance.

Results

Parasites inventory

Microscopic examination of the 32 grebes revealed four ectoparasite species: two feather mite species from the suborder Astigmata (superfamily Pterolichoidea, family Ptiloxenidae) and two louse species representing two different families, Philopteridae and Menoponidae.

Mites were the most abundant and diverse arthropods in the Grebes plumage, with 8,033 individuals recorded. These mites were present in most examined birds, exhibiting the highest prevalence and mean intensity among all identified ectoparasites.

Two species of feather mites were recorded: *Ptiloxenus major* Mégnin and Trouessart, 1884 (Fig. 3) and *Ptiloxenus colymbi* Canestrini, 1878 (Fig. 4). *P. major* was the most abundant species, with 7,338 individuals. It exhibited a prevalence of 100% in both host species, underscoring its wide distribution within the examined

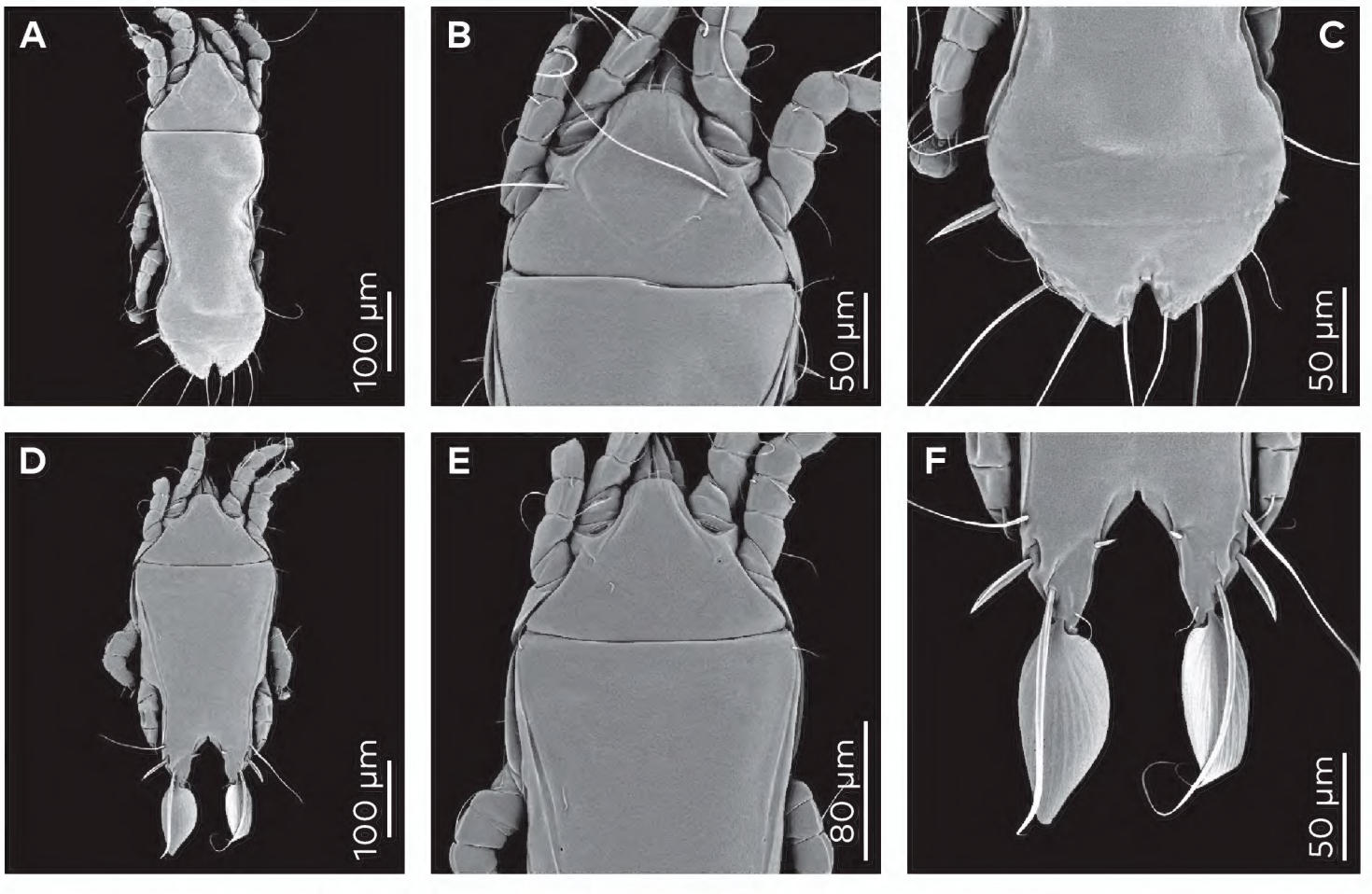

Figure 3. *Ptiloxenus major*, SEM images: **A–C** Details of female idiosoma; **D–F** Details of male idiosoma.

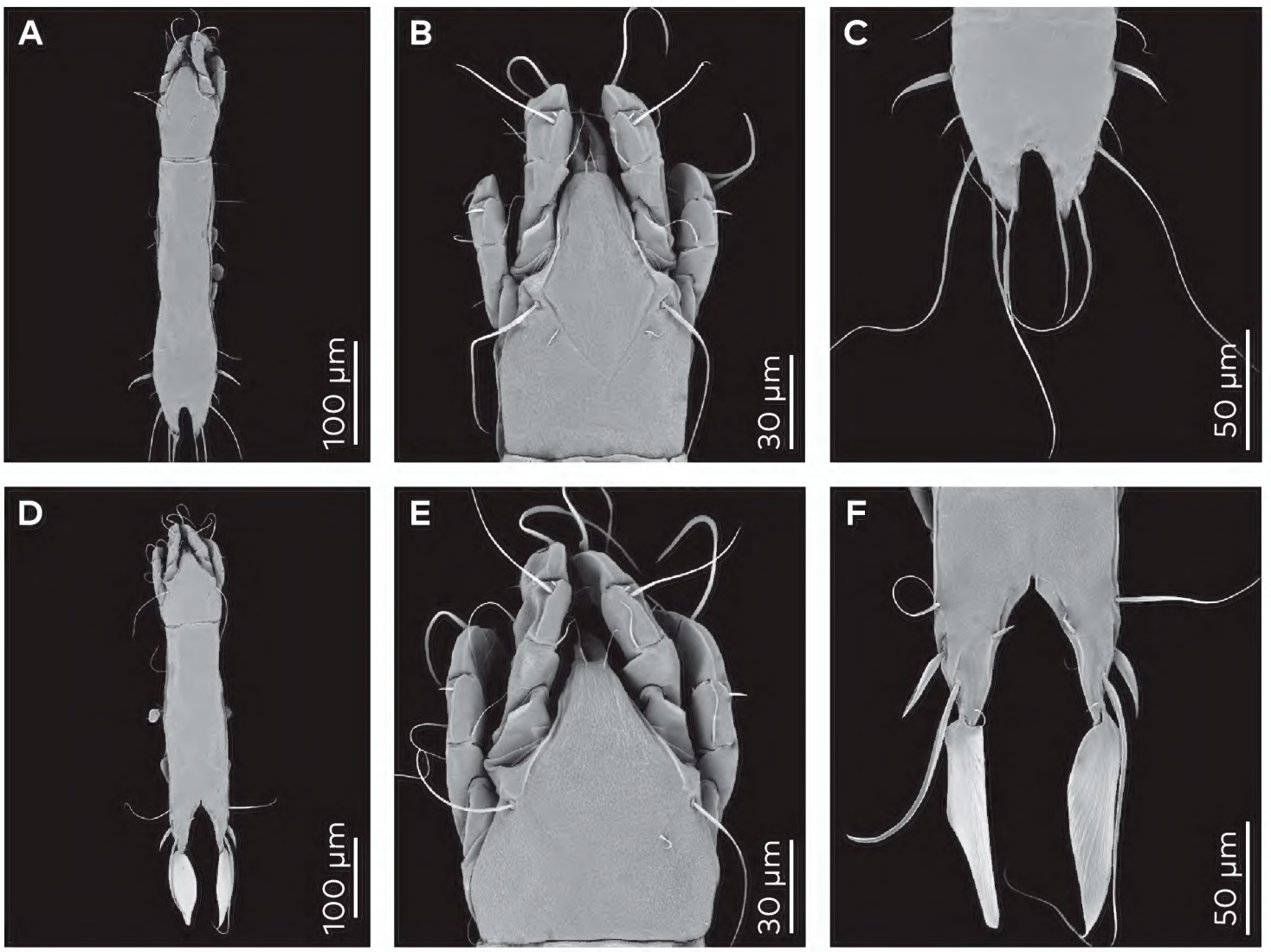

Figure 4. *Ptiloxenus colymbi*, SEM images: **A–C** Details of female idiosoma; **D–F** Details of male idiosoma.

Grebe populations. Furthermore, it demonstrated a notably high mean intensity, with values of 445.53 in the Great Crested Grebe and 81.36 in the Little Grebe, indicating substantial mite loads per infested individual.

In contrast, *P. colymbi* was represented by 695 individuals, 406 from the Great Crested Grebe and 289 from the Little Grebe, corresponding to 38% and 63% prevalence rates, respectively. The mean intensity values for this species were 81.2 and 24.08 in the Great Crested Grebe and Little Grebe, respectively.

Regarding lice, two species were identified: *Aquanirmus podicipis* Denny, 1842 (11♀, 8♂, four nymphs) from the Great Crested Grebe and *Pseudomenopon dolium* (Rudow, 1869) (one ♂) from the Little Grebe (Fig. 5). This study represents the first record of these mites and lice species in Algeria. Interestingly, the sex ratio of all analyzed ectoparasite species was biased in favor of females. A summary of all identified ectoparasite species, including their prevalence, mean abundance, and mean intensity, is provided in Table. 1.

Statistical analyses

Due to the limited number of lice collected through visual examination, which likely underestimates true infestation levels, statistical analyses could not be performed

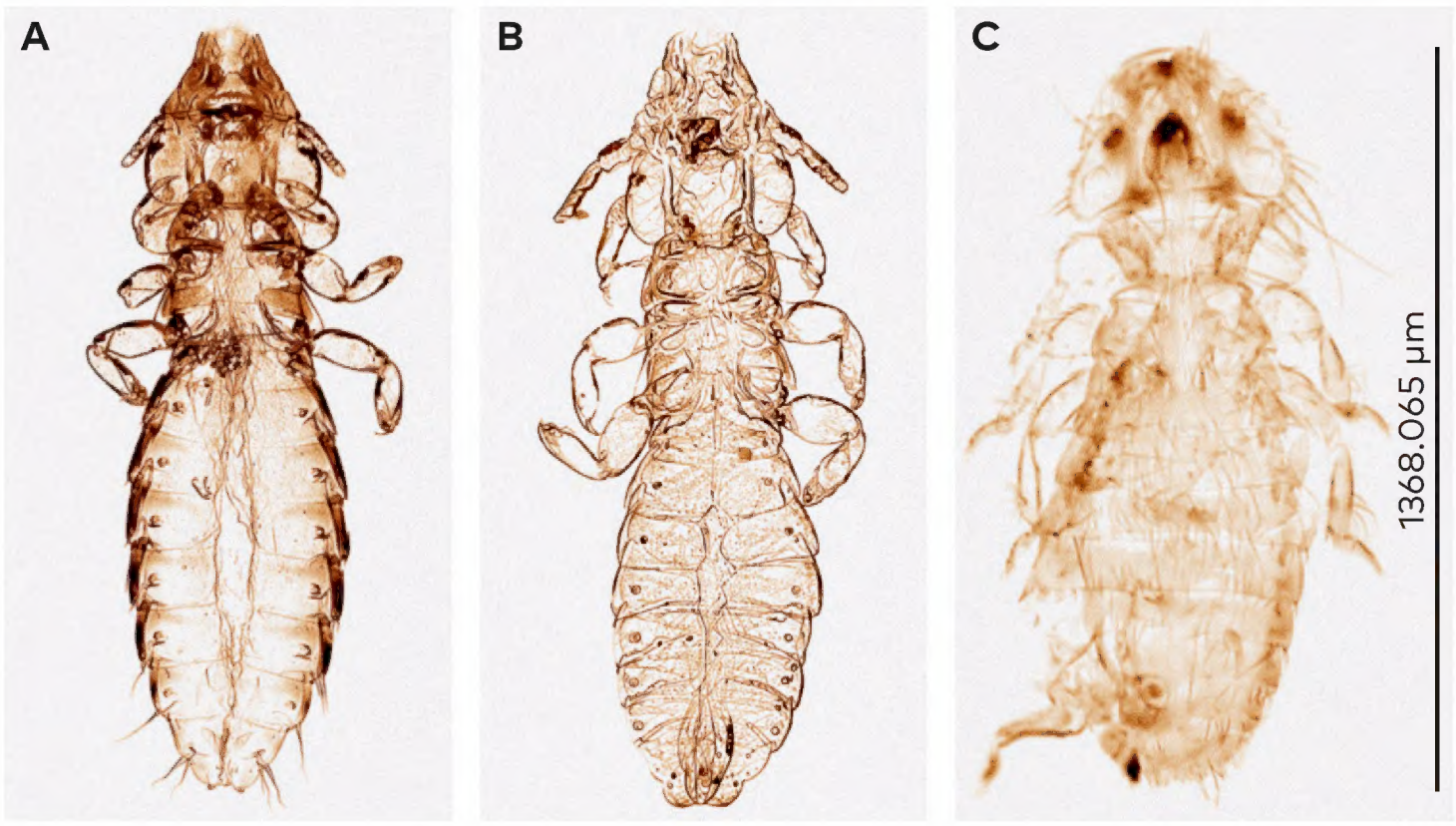

Figure 5. Lice identified on Little Grebe and Great Crested Grebe: **A** female; **B** male of *Aquanirmus podicipis*; **C** male of *Pseudomenopon dolium* from Lake Tonga (Algeria).

for lice. In contrast, sufficient feather mite samples were obtained, allowing us to perform statistical analyses.

Table 2 presents the distribution of feather mites across different body parts of grebes and shows that the wings are the primary site of infestation in both species. In the Great Crested Grebe, the majority of feather mites (93.06%) were recorded on the wings, followed by lower proportions on the back (5.67%), belly (1.08%), and head and neck (0.19%). A similar pattern was observed in the Little Grebe, where 90.79% of feather mites occurred on the wings, with additional presence on the back (7.19%), belly (1.86%), and head and neck (0.16%). Overall, these results indicate a strong preference of feather mites for the wings, while other body regions hosted only minimal infestations.

Table 1. Parasites identified on Little Grebe and Great Crested Grebe from Lake Tonga (Algeria).

Host	Ectoparasites	Infested birds (N)	Parasite number (N)	Prevalence (%)	Mean abundance ± SD	Mean intensity ± SD	Male	Female	Nymph	Sex ratio
Great Crested Grebe	*Ptiloxenus major*	13	5792	100%	445,53±13.65	445,53±35.48	2189	2837	766	0.77
	Ptiloxenus colymbi	5	406	38%	31,23±1.73	81,2±4.51	139	172	95	0.81
	Aquanirmus podicipis	7	23	53%	1,76±0.23	3,28±0.6	5	11	7	0.45
Little Grebe	*Ptiloxenus major*	19	1546	100%	81,36±2,42	81,36±3,82	711	591	244	1.20
	Ptiloxenus colymbi	12	289	63%	15,21±1.10	24,08±1.74	84	114	91	0.74
	Pseudomenopon dolium	1	1	5%	0,05±0.01	1±0.23	1	0	0	0

Table 2. Distribution of feather mites across different body parts of host species.

Bird species	Head and neck	Belly	Back	Wings
Great Crested Grebe	0,19%	1,08%	5.67%	93.06%
Little Grebe	0.16%	1.86%	7.19%	90.79%

In this study, the seasonal variation of feather mite infestations in the Little Grebe and Great Crested Grebe during the breeding and wintering seasons were examined. Figure 6 provides a visual representation of feather mite infestation across the two seasons. Feather mite abundance is markedly higher during the breeding season, with a broader range and a higher median compared to wintering individuals. A similar, though less pronounced, pattern is observed in Little Grebe, where mite loads are also elevated during the breeding season and considerably reduced in winter. Despite the clear visual trend of higher mite loads in the breeding season for both species, statistical analyses failed to show a statistically significant difference between the breeding and wintering seasons for either species (p values > 0.05) (Table 3).

The Principal Component Analysis (PCA) revealed that feather mites is weakly correlated with the morphometric parameters which form a tightly grouped cluster along Dim 1. While these morphometric measures are strongly positively correlated with each other, feather mites stands apart, contributing little to the main variance structure and displaying only a modest association with Dim 2. This separation indicates that feather mite abundance varies largely independently from the morphometric traits, highlighting a low correlation between feather mites and all measured morphometric parameters (Fig. 7).

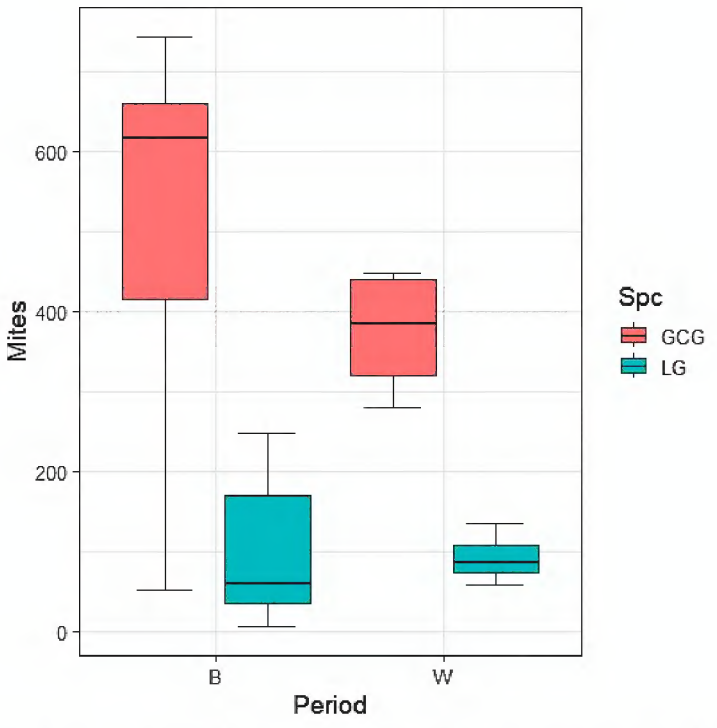

Figure 6. Boxplots depicting seasonal variation in feather mite infestation in Great Crested Grebe and Little Grebe populations.

Table 3. Statistical influence of the seasons on feather mite infestation in Great Crested Grebe and Little Grebe populations.

Saison	Little Grebe	Great Crested Grebe
Breeding (mean ± se)	101.778±30.45	521.889±72.841
Wintering (mean ± se)	91.9±8.267	375.25±40.978
Normality test *p-value*	0.1141	0.4791
t-test *p-value*	0.7612	0.1073

Discussion

This study represents the first investigation of the ectoparasite fauna of the Little Grebe and the Great Crested Grebe in Lake Tonga, northeastern Algeria. Given that birds serve as hosts for specific ectoparasites, identifying the parasite species present on grebes is essential for understanding host-parasite dynamics and their role in structuring avian communities. Based on morphological analysis, this study recorded new ectoparasite species for Algeria. Regarding feather mites, two species were identified, both belonging to the family Ptiloxenidae Gaud, 1982 and the genus *Ptiloxenus* Hull, 1934, which is exclusive to Podicipediformes (Dubinin 1956; Gaud and Atyeo 1996; Ebermann 1998).

Ptiloxenus major was originally described from specimens collected from the Great Crested Grebe and the Red-Throated Diver in Europe. It has been reported on various grebe species, including the Red-Necked Grebe, Horned Grebe, Black-Necked Grebe, Silvery Grebe, White-Tufted Grebe, and Little Grebe, across multiple geographic locations, including Switzerland, the Baraba Steppe, Germany, Argentina, Paraguay, Czechoslovakia, Transvaal, Canada (Alberta and Manitoba) (Mironov 1997; Dubinin 1951, 1956; Černý 1971; Bedford 1932; Ebermann 1998; Černý 1961; Dabert and Ehrnsberger 2003; Galloway et al. 2014).

Similarly, *Ptiloxenus colymbi* was initially described from the Little Grebe in Europe without accompanying illustrations and was later reported in Cameroon, Germany, Russia (Gaud and Mouchet 1959; Dubinin 1956; Ebermann 1998; Zumpt et al. 1961). Since these early records, no further documentation of *P. colymbi* has been made. In the present study, this species was detected on both grebe species, representing a new host-parasite association in global mite fauna. This association is considered ecologically plausible, as both species belong to the Podicipedidae family and are resident breeders in the study area (Elafri et al. 2017). Their shared breeding and feeding grounds likely facilitate the transfer of mites between individuals (Welty and Baptista 1990). Notably, each bird was examined individually to prevent cross-contamination of samples.

Two different louse species were collected from grebes in this study: *Aquanirmus podicipis* and *Pseudomenopon dolium*. The former was recovered exclusively from the Great Crested Grebe, whereas the latter was found only on the Little Grebe. Similar

patterns have been reported elsewhere; in Türkiye; both species were detected on the Great Crested Grebe (Dik et al. 2017), and their presence has also been documented in Iran (Dik and Halajian 2013). In addition, *P. dolium* has been recorded in Chile from the Southern Silvery Grebe (González-Acuña et al. 2017). Our results are therefore consistent with previous studies and contribute new regional data on the lice associated with grebes.

The lack of records of *Pseudomenopon dolium* from Great Crested Grebe in our study is likely due to the difficulties of collecting this species manually, as it normally occurs among the dense body feathers. Neither *Aquanirmus bahli*, nor *A. runcinatus*, known from different subspecies of *T. ruficollis* (Price et al. 2003) were collected in our study. The hypothesis of Edwards (1965) that the host associations of *T. ruficollis* and *Aquanirmus spp*. differ between Europe and Africa+Asia, with the former resulting from secondary infestation, could therefore not be tested in this study. More data are needed from Little Grebe across its range to test this hypothesis. Data from Algeria may be especially interesting, given its location between the Europan range of *A. runcinatus* and the Afro-Asian range of *A. bahli* (Edwards 1965).

Table 1 shows that the prevalence of lice was lower than that of feather mites, with an overall prevalence of 5% for *P. dolium* and 53% for *A. podicipis*. This discrepancy can be attributed, on the one hand, to the sampling method, as the number of species collected from living birds is often underestimated when fumigation is not used. Additionally, the number of lice on a bird varies among host taxa, often correlating with its size and possibly its social system. On the other hand, preening behavior plays a crucial role in limiting louse abundance, which explains their reduced numbers (Galloway and Lamb 2021; Clayton 1991).

The preference of feather mites for the wings can be explained by several factors. Wings provide a dense and structurally complex microhabitat that offers protection

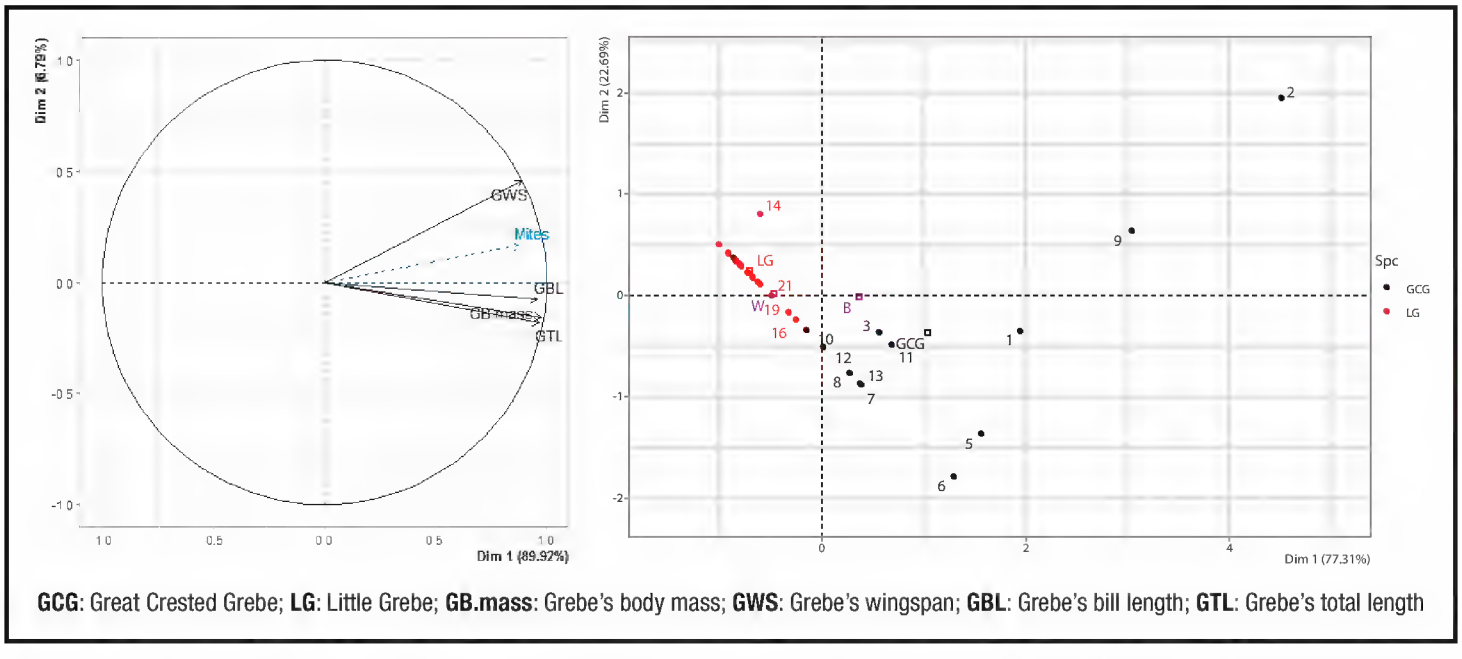

Figure 7. The Principal Component Analysis (PCA) biplot, illustrating the influence of morphometric parameters on feather mite infestation in grebes.

from environmental stressors and predation, while also limiting the host's ability to groom effectively.

Seasonal variations in feather mite infestations, with higher loads typically during the breeding season, can be attributed to various factors. Increased grebe population density during the breeding season, ranging from 499 to 608 individuals (Mecif and Boucheker 2020; Loucif et al. 2020), likely facilitates parasite transmission. However, in our study these differences were not statistically significant, which may reflect an insufficient sample size or the influence of additional, unmeasured factors.

Feather mites showed a low correlation with morphometric parameters, it may be influenced more strongly by factors unrelated to host body size or morphology, such as behavior, microhabitat preferences, immune responses, or environmental variables.

Overall, this finding highlights the complexity of host-parasite interactions and the need for more extensive sampling and to consider additional variables beyond season and morphology when studying feather mites ecology.

Conclusion

Research on aquatic birds in Algeria remains limited, particularly concerning chewing lice and feather mites, as well as their role in shaping host communities. Further investigations into the parasite fauna of aquatic birds are essential to assess their ecological impact, the diseases they may transmit, and their influence on host populations. Future studies should integrate seasonal dynamics, morphometric parameters, and environmental factors to enhance our understanding of host-parasite interactions and their broader implications for ecosystem dynamics.

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